

## **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

### **Listing of Claims:**

1-115. **(Canceled)**

116. **(Previously presented)** A system for control of gene expression comprising:

(i) a first nucleic acid molecule comprising a cis-repressive sequence element upstream of an open reading frame (ORF), or including part of the open reading frame, wherein the first nucleic acid molecule forms a stem-loop structure that represses translation of the ORF; and

(ii) a second nucleic acid molecule comprising first and second stem-forming portions and a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion to form a loop, and wherein a portion of the second nucleic acid molecule is complementary or substantially complementary to a portion of the first nucleic acid molecule and interacts with the first nucleic acid molecule to derepress translation of the ORF.

117-176. **(Canceled)**

177. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element;

and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element; and

(c) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

178. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:

a plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

179. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element;

and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element;

(c) a third plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

(d) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

180. **(Previously presented)** A kit comprising:

one or more oligonucleotides comprising a crRNA sequence, one or more oligonucleotides comprising a taRNA sequence, or one or more oligonucleotides comprising a crRNA sequence and one or more oligonucleotides comprising a taRNA sequence, wherein the kit further comprises one or more items selected from the group consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

181. **(Withdrawn)** A method of regulating translation of an open reading frame comprising steps of:
- introducing an engineered template for transcription of an mRNA into a cell and allowing mRNA transcription to occur resulting in a transcribed mRNA, wherein the template is engineered so that the transcribed mRNA comprises first and second nucleic acid elements that form a stem-loop structure that represses translation of the mRNA; and
  - providing an engineered nucleic acid molecule that interacts with the mRNA so as to derepress translation of the mRNA to the cell.
182. **(Withdrawn)** The method of claim 181, wherein the engineered template comprises:
- (i) a first stem-forming portion;
  - (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary;
  - (iii) a non-stem-forming portion connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion; and
  - (iv) an open reading frame (ORF),
- wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation of the ORF.
- 183-242. **(Canceled)**
243. **(New)** The method of claim 181, wherein the engineered nucleic acid molecule comprises:
- (i) a first stem-forming portion;
  - (ii) a second stem-forming portion; and
  - (iii) a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem forming portion and the 5' end of the second stem-forming portion to form a loop,
- and wherein a portion of the nucleic acid molecule is complementary or substantially complementary, to a portion of the transcribed mRNA.

244. **(New)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 80%.
245. **(New)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 90%.
246. **(New)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 98%.
247. **(New)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 5 fold.
248. **(New)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 10 fold.
249. **(New)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 19 fold.
250. **(New)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of RNA.
251. **(New)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA.
252. **(New)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA and RNA.
253. **(New)** The system of claim 116, wherein the nucleic acid molecule is positioned upstream of the ORF.
254. **(New)** The system of claim 116, wherein the first nucleic acid molecule comprises:
- (i) a first stem-forming portion;

(ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary, and

(iii) a non-stem-forming portion that forms a loop connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion, wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation when positioned upstream of an open reading frame (ORF).

255. **(New)** The system of claim 254, wherein the first and second stem-forming portions are substantially complementary.
256. **(New)** The system of claim 116, wherein at least a portion of the first nucleic acid molecule is complementary or substantially complementary to a ribosome binding site (RBS).
257. **(New)** The system of claim 116, wherein at least a portion of the first nucleic acid molecule is complementary or substantially complementary to a Kozak consensus sequence.
258. **(New)** The system of claim 254, wherein the sequence of the second stem-forming portion comprises an RBS.
259. **(New)** The system of claim 254, wherein the sequence of the non-stem-forming portion comprises YUNR.
260. **(New)** The system of claim 254, wherein the non-stem forming portion is 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides in length.
261. **(New)** The system of claim 254, wherein the non-stem forming portion is between 13 and 50 nucleotides in length, inclusive.
262. **(New)** The system of claim 116, whereby the length of the stem is between 4 and 100 nucleotides, inclusive.

263. **(New)** The system of claim 116, wherein the length of the stem is between 6 and 50 nucleotides, inclusive.
264. **(New)** The system of claim 116, wherein the length of the stem is between 12 and 30 nucleotides, inclusive.
265. **(New)** The system of claim 116, wherein the length of the stem is approximately 19 nucleotides .
266. **(New)** The system of claim 116, wherein the stem exhibits at least 66% complementarity.
267. **(New)** The system of claim 116, wherein the stem exhibits between 75 and 95% complementarity.
268. **(New)** The system of claim 116, wherein the stem exhibits approximately 85% complementarity.
269. **(New)** The system of claim 116, wherein the stem includes at least one area of non-complementarity.
270. **(New)** The system of claim 269, wherein the stem includes at least one bulge.
271. **(New)** The system of claim 116, wherein the stem includes at least two dispersed areas of non-complementarity.
272. **(New)** The system of claim 271, wherein the stem includes at least two dispersed bulges.
273. **(New)** The system of claim 116, wherein the stem includes at least three dispersed areas of non-complementarity.
274. **(New)** The system of claim 273, wherein the stem includes at least three dispersed bulges.
275. **(New)** The system of claim 116, wherein the nucleic acid molecule forms a single stable stem.

276. (New) The system of claim 116, wherein the nucleic acid molecule represses translation in the absence of a ligand.
277. (New) The system of claim 116, wherein the first stem-forming portion comprises a sequence complementary or substantially complementary to a sequence in the 5' portion of an ORF.
278. (New) The system of claim 254, wherein the nucleic acid molecule comprises a start codon.
279. (New) The system of claim 278, wherein the nucleic acid molecule comprises a spacer comprising one or more nucleotides between the 3' end of the second stem-forming portion and the start codon.
280. (New) The system of claim 278, wherein all or part of the start codon is located within the second stem-forming portion.
281. (New) The system of claim 116, wherein the nucleic acid molecule comprises one or more nucleotides at the 5' end that do not participate in the stem-loop structure.
282. (New) The system of claim 116, wherein the nucleic acid molecule comprises between 5 and 50 nucleotides upstream of the 5' end of the first stem-forming portion.
283. (New) The system of claim 116, wherein the nucleic acid molecule comprises a ligand binding domain.
284. (New) The system of claim 254, wherein the nucleic acid molecule comprises a third stem-forming portion that is complementary or substantially complementary to the second stem-forming portion, wherein the first and third stem-forming portions form alternate stem-loop structures with the second stem-forming portion.
285. (New) The system of claim 284, wherein the first and third stem-forming portions comprise a portion that is complementary or substantially complementary to an RBS.



286. **(New)** The system of claim 116, wherein the second nucleic acid molecule comprises a portion comprising the sequence YNAR positioned 5' to the 5' portion of the first stem-forming sequence.
287. **(New)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 6 and 50 nucleotides.
288. **(New)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 12 and 30 nucleotides.
289. **(New)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is approximately 19 nucleotides.
290. **(New)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit at least 66% complementarity.
291. **(New)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit between 75 and 95% complementarity.
292. **(New)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit approximately 85% complementarity.
293. **(New)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least one area of non-complementarity.
294. **(New)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least two dispersed areas of non-complementarity.
295. **(New)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least three dispersed areas of non-complementarity.

296. **(New)** The system of claim 116, wherein the second nucleic acid molecule comprises a nucleotide analog.
297. **(New)** The system of claim 116, wherein the second nucleic acid molecule comprises a ligand binding domain.
298. **(New)** The system of claim 116, wherein the first and second nucleic acid molecules interact so as to disrupt the stem-loop structure formed by the first nucleic acid molecule, thereby allowing a ribosome to gain access to a ribosome binding site.
299. **(New)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 and the second nucleic acid molecule has the sequence of taR10.
300. **(New)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 and the second nucleic acid molecule has the sequence of taR12.
301. **(New)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 or a variant of crR10 that differs from crR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR10 or a variant of taR10 that differs from taR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.
302. **(New)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 or a variant of crR12 that differs from crR12 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR12 or a variant of taR12 that differs from taR12 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.
303. **(New)** The system of claim 116, wherein the first nucleic acid molecule and the second nucleic acid molecule have an equilibrium association constant between  $0.8 \times 10^7$  and  $1.5 \times 10^7$  kcal/mol.